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FERTILIZATION AND EMBRYOGENY IN CEPHALOTAXUS FORTUNEI

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(WITH FIVE FIGURES AND PLATE I)

The material for this study was collected at Darlington, S. C., where the plant grew normally and produced good seeds. Collections of young ovules were also made in Bonn, Germany, but they are here represented only in *fig. 1*.

Our knowledge of the spermatogenesis of Cephalotaxus is confined to the work of STRASBURGER (18) and of ARNOLDI (1). Twenty-seven years ago STRASBURGER described the development of the embryo quite accurately for the stages observed, but he did not secure young proembryos. ARNOLDI has described the gametophytes and proembryo, but he has overlooked certain interesting peculiarities and is in places not sufficiently clear.

Material is not yet at hand to determine the development of the young ovule in detail, and its early history will not be considered here. However, one figure is given (*fig. 1*) from material collected January 5, 1902, at Bonn, to show the interesting midwinter condition of the ovule. This is about nine and one-half months after pollination and the pollen tube has developed into a large sac which occupies a great part of the tip of the nucellus. The body cell and two vegetative nuclei are noticed near the center. In the massive lower part of the ovule is the megaspore, not yet divided. This winter condition will at once suggest the great difference between the genera of the Taxeae in regard to the time elapsing between the critical points of pollination, fertilization, and maturation of the seeds. In both Torreya (COULTER and LAND, 6) and Cephalotaxus the

time between pollination and maturity of the seed is about eighteen months, but the relative length of time between pollination and fertilization, and fertilization and maturity is reversed in the two genera. In Torreya fertilization follows in about four months after pollination, while in Cephalotaxus about fourteen months elapse between the two events. We find, therefore, that most of the growth of the ovule follows fertilization in Torreya, and precedes it in Cephalotaxus.

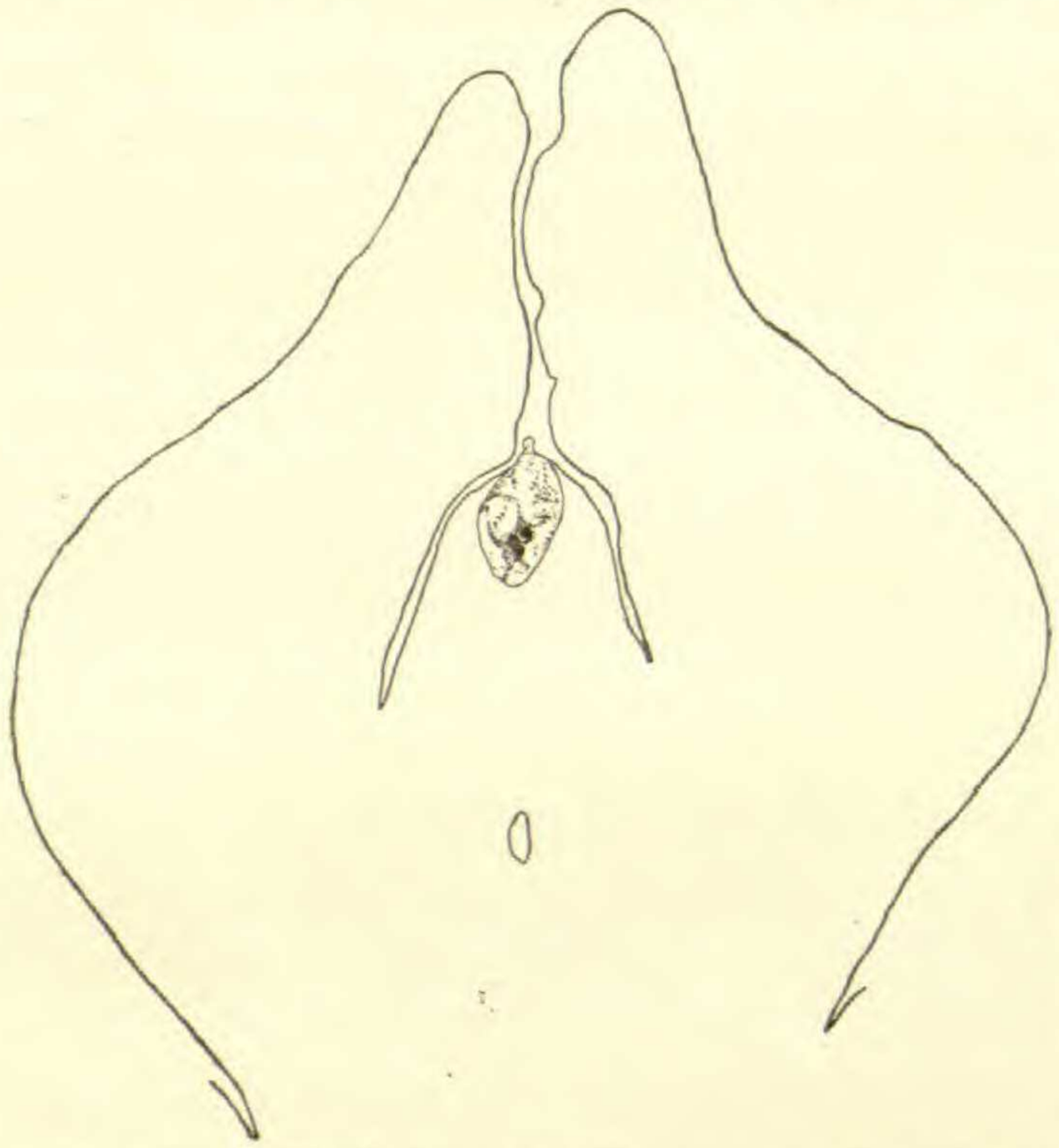

FIG. 1.—Pollinated ovule in winter condition. Bonn, Germany, Jan. 5. 1902. ×45.

The ovule of Cephalotaxus at the time of fertilization is quite large, over 1.5^{cm} long, and the prothallium is well developed; while at the same stage, the ovule of Torreya is only about one-fifth as long, although at maturity it is much larger than the ovule of Cephalotaxus. In Taxus the ovule matures in one growing season, fertilization following pollination in about two months. As a result of this very rapid sequence we find that at the time of fertilization its prothallium is extremely small and delicate—more so than in any other gymnosperm, with the possible exception of Torreya.

The pollen grain of Cephalotaxus divides before being shed (STRASBURGER, **19**) and there are no prothallial cells formed. The tube contents show the normal structure, the tube and stalk nuclei lying just in front of the body cell (*fig. 1*). ARNOLDI states that in "manchen Fallen" one can make out three nuclei in addition to the body cell in the tip of the pollen tube, and adds that, as regards the contents of the pollen tube, Cephalotaxus may be distinguished from

all other conifers. These observations I cannot confirm, as all the pollen tubes examined had the usual two vegetative nuclei as in Taxus, Torreya, and other Coniferae.[1]

In the spring of its second season the pollen tube still grows very slowly, remaining broad and sac-like until about two or three weeks before fertilization, when it rapidly penetrates the short distance to the female prothallium and spreads its tip over the neck of an archegonium. The body cell which has been growing slowly all the time now shows the structure indicated in *fig. 2*.[2] Its protoplasm is very dense and shows well-marked radiations from a denser point just below the nucleus. The nucleus occupies a very eccentric position near the upper side of the cell, thus indicating the unequal division that is to follow.

When the tube becomes flattened out on the neck of the archegonium, the stalk and tube nuclei may be touching or slightly pressed into the body cell; usually, however, they are at a greater distance from it.

A few days before fertilization the body cell divides into two sperm cells of unequal size, the lower being in every case the larger. The difference in size is not so great as in *Torreya taxifolia* (COULTER and LAND, **6**) or in Taxus (BELAJEFF, **3**) but it is nevertheless decidedly constant. In the seventeen pollen tubes that appeared in this stage in my preparations, the difference in size of the two sperm cells could in every case be easily made out. In two of three supernumerary tubes that had been left over after fertilization, the nucleus of the smaller cell had grown larger than the other, thus obscuring to some extent the unequal distribution of the protoplasm. In *figs. 3, 4, 5* are shown three pairs of sperm cells of unequal size. It will be seen that each sperm cell is quite distinct from its fellow and from the protoplasm of the pollen tube. Soon after their formation the smaller cell tends to round itself more quickly than the larger (*fig. 5*). Another indication that the two sperm cells are not of equivalent value is that the nucleus of the smaller may not be so dense as that of the larger (*fig. 4*).

[1] It is probable that ARNOLDI'S material varied at times from the normal, as I suppose also to have been the case in my material of Podocarpus, where three vegetative nuclei were found in the pollen tube.

[2] For *figs. 2–17*, see PLATE I.

Each pollen tube applies itself to but one archegonium, and in fertilization only the forward and larger of the two sperm cells is functional. ARNOLDI (1) in his study of Cephalotaxus does not mention any difference in size between the two sperm cells. He probably overlooked it. In *Torreya taxifolia* COULTER and LAND find that the two sperm cells are very unequal and that, as in Cephalotaxus, each is distinct from the other. In *Torreya californica*, however, Miss ROBERTSON (17) cannot distinguish any division of the protoplasm between the two sperm nuclei. She says: "these two nuclei seem never to be surrounded by separate masses of cytoplasm, agreeing in this respect with Pinus." She finds that the two nuclei are of equal size and believes that only one of them is functional.

In *Taxus baccata*, according to BELAJEFF (3), STRASBURGER (19), and JAEGER (8),[3] the very unequal sperm cells are quite distinctly separated, and JAEGER finds their nuclei of equal size.

In my work on Podocarpus (4) I found that only one functional sperm cell was formed, one nucleus being thrust to the surface of the cell. In the two preparations showing this stage, this nucleus did not seem to have any separate protoplasm of its own, but I would not be sure of this without further investigation.

We find then, in summarizing our present knowledge, that all the Taxaceae so far investigated show but one functional sperm cell, and that in the Taxeae proper (with the exception of *Torreya californica*) there are two distinct sperm cells of unequal size, the difference being more pronounced in Taxus and Torreya than in Cephalotaxus.

In Pinus (Miss FERGUSON, 7), Picea (MIYAKE, 12), and Abies (MIYAKE, 13), the two sperm nuclei lie in a common protoplasm, but the forward one is the larger and alone is functional. According to MURRILL'S (14) account one would suppose that in Tsuga the two sperm cells are distinct, but the nucleus of the forward functional cell is decidedly larger, as in the other Abieteae above mentioned. It would seem, therefore, that in all gymnosperms whose pollen tubes fertilize but a single archegonium there is but one functional sperm cell (Taxaceae) or sperm nucleus (Abieteae).[4]

[3] JAEGER'S *fig. 34* shows a distinct line between the two cells, but he says: "Die Umrisse der kleinen generativen Zelle, die von Archegonium abgekahrt ist, erkennen wir nur undeutlich, sie ist nicht so stark gefärbt, wie die grosse generative Zelle."

[4] The peculiar multiplication of the number of sperm cells found in conservatory grown material of *Cupressus Goveniana* by JUEL (Flora **93**:56–62. *pl. 3.* 1904) is probably abnormal.

The archegonia of Cephalotaxus vary in number from two to five; three is a common number in my preparations. They are always situated in the micropylar end of the prothallium and are never in contact with each other for any distance and only rarely touch each other at any point. They are extremely long in comparison with their width, and are sharply pointed below. The usual shape is represented in *fig. 12*. The one shown in *fig. 9* is shorter than usual. The jacket is not nearly so well-developed as in some other conifers, and is frequently interrupted by ordinary cells (*fig. 12*).

According to ARNOLDI there are two neck cells (he mentions no exception), but in one case I found as many as five neck cells and in several cases three and four, all in one plane. In *fig. 6* a neck of three cells is shown from above. Occasionally the tip of the archegonium pushes beyond the neck cells, moving them to one side (*fig. 7*). Up to ten or fifteen days before fertilization the archegonia have very little protoplasmic contents. The nucleus at this time is small and is very close to the upper end. The protoplasm begins to thicken rapidly just before the division of the central cell, which occurs about ten days before fertilization. The ventral canal nucleus is without any distinctive protoplasm of its own, as ARNOLDI has already pointed out, resembling in this respect that of Podocarpus (COKER, **4**), Taxodium (COKER, **5**), Cryptomeria (LAWSON, **11**), Thuja (LAND, **9**), and Juniperus (NORÉN, **15**). Miss ROBERTSON found the spindle of the division in *Torreya californica*, but no later stages. In *fig. 8* is shown the ventral nucleus and the egg nucleus soon after the division. The former is at the upper surface of the protoplasm. In *fig. 9* the canal nucleus is shown in its usual position, but there is the curious abnormality of two other nuclei in the egg. The canal nucleus sometimes moves away from the surface and approaches nearer the egg nucleus (*fig. 10*), but this must be considered an abnormality. The canal nucleus generally disappears before fertilization and cannot be demonstrated at that time.

In Darlington, S. C., fertilization took place, in 1903, from the fifth to the eighth of May. The necks of the archegonia are at this time at the bottom of the pits formed by upgrowths of the prothallium. The pollen tubes reach the archegonia before these pits are formed and the advancing prothallium grows around them. In case

there is no pollen tube above an archegonium, the growth of the prothallium may close the pit and bury the archegonium completely.

Both sperm cells are discharged into the egg, but the two vegetative nuclei may remain behind. The larger sperm cell advances to the egg nucleus, the sperm nucleus sinks into it, and the sperm protoplasm gradually surrounds the fusing nuclei, exactly as I have already described for Taxodium. *Figs. 11, 12, 13, 14* show stages of approach and fusion. The second sperm cell remains above (*figs. 11, 12*) and may approach pretty close to the fusion nucleus (*fig. 14*). ARNOLDI (1) says that soon after the sperm cells enter the archegonium, the protoplasm becomes mixed with that of the egg. He does not observe the sperm protoplasm investing the fusion nucleus.

The contribution of the sperm protoplasm to the proembryo has also been observed in Torreya by Miss ROBERTSON (17) and COULTER and LAND (6), in Cryptomeria by ARNOLDI (1) and LAWSON (11), in Juniperus by NORÉN (15), and in Sequoia by ARNOLDI (2). In LAWSON'S (10) work on Sequoia he does not confirm ARNOLDI, but finds that only a very small amount of the sperm protoplasm enters the egg with the nucleus, the rest remaining behind in the pollen tube. This odd behavior is similar to the process of fertilization in Taxus as described by BELAJEFF (3). There is no starch in the sperm cells of Cephalotaxus such as is found in Taxodium, Sequoia, Cryptomeria, and Juniperus.

The first division of the fusion nucleus occurs near the center of the archegonium. The spindle is very small compared with the size of the nucleus and is entirely intranuclear. *Fig. 14* shows an early stage in this division. The sperm protoplasm has not yet entirely invested the nucleus. In *fig. 15* the division is complete and the two nuclei are approaching the base of the archegonium. Two extra nuclei are shown above—one is probably the second sperm nucleus. The next division occurs before the proembryo has reached the base of the archegonium. Three of the four nuclei produced by this division are shown in *fig. 16*.

In *fig 17* is shown the eight cell-stage which now occupies the base of the archegonium. Five of the eight nuclei are in the section. These eight nuclei now divide again simultaneously, and after the formation of the sixteen daughter nuclei, cell walls are formed for

the first time. *Fig. 18* shows this stage just at the beginning of the cell wall formation. The cells are not arranged in regular tiers throughout, being most irregular in the central region. At the upper end are two fairly even tiers, and the tip is occupied nearly always by two superimposed cells. In archegonia with more rounded bases, which appear occasionally, there may be two cells side by side in the tip.

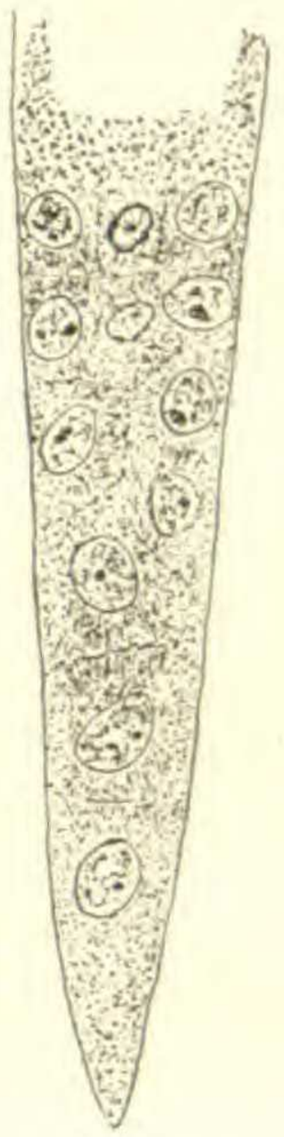

FIG. 18.—Sixteen celled proembryo with cell walls beginning to form. ×185.

After the formation of cell walls the sixteen cells divide again to form thirty-two, and it seems probable that the upper tier divides horizontally to form the rosette cells and suspensors. I have not found any case, however, where the rosette consists of free nuclei, as is so common in conifers. The cells of the more or less regular tier above the suspensors are enclosed in the cell walls, and occasionally one or more of them may divide horizontally. In *fig. 19* the thirty-two cell-stage is represented. The upper tier (*r*), which answers to the rosette of five cells; the next tier (*s*), which forms the suspensors, also contains five cells; while the remaining cells are not in regular tiers except near the tip, where there are three distinct tiers, the upper of two cells, the two lower of one cell each.

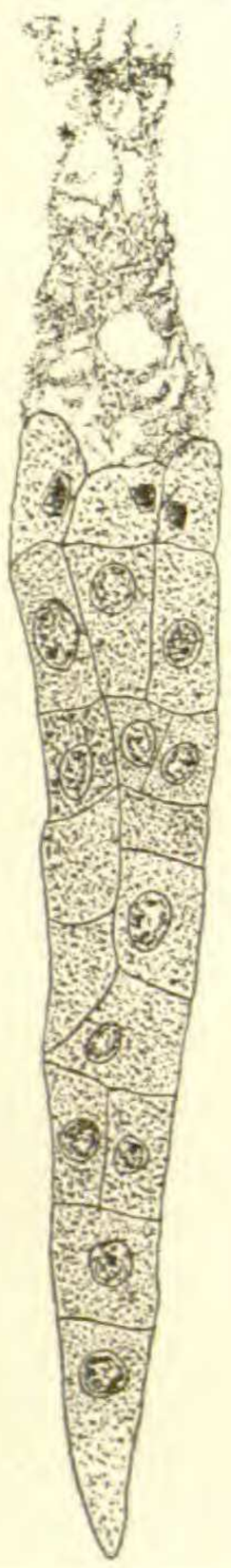

FIG. 19.—Thirty-two-celled proembryo just before the elongation of suspensors. ×185.

The suspensors now begin to elongate and cell divisions occur in any of the cells of the middle region below the suspensors and above the two tip cells. These divisions are no longer simultaneous, but occur here and there as in ordinary growth.

In *fig. 20* is shown an embryo in which the suspensors have just begun to elongate. One cell of the upper tier has divided into two, and the number of cells in the middle region has increased somewhat. The two tip cells show

no signs as yet of the disorganization that, according to STRASBURGER (18), they are to undergo. The protoplasm of the tip cell is somewhat less dense than the others, but not markedly so. The nuclei of this cell and the one above it, however, are much larger than the others, and the cells give every indication of being actively secretive. At this stage they have been driven some distance downward into the prothallial tissue, but instead of being crushed by the growth behind they seem to be opening the way for the progress of the embryo. As I have not yet secured stages immediately following this, I cannot determine the ultimate fate of the tip cells. It is certain, however, that they persist for some time in healthy and active condition at the tip of the embryo.

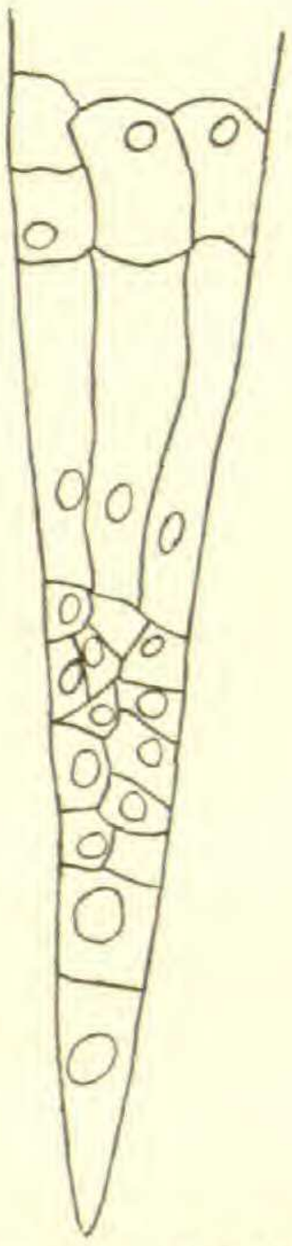

FIG. 20.—Slightly older proembryo; suspensors elongating. May 19, 1903. ×150.

A much older embryo is represented in *fig. 21*. Above the crumpled suspensors and embryonal tubes three abortive embryos are seen. These are probably the products of three additional archegonia, as it certainly is not the normal thing for an archegonium to produce more than one embryo in Cephalotaxus. In none of the proembryos seen did the suspensors show signs of separating, as they do in many other conifers, and from the structure of the proembryo this does not surprise us.

FIG. 21.—Older embryo with abortive ones above. June 16, 1903. ×45.

ARNOLDI (1) does not state definitely the number of cell divisions before the formation of walls in the proembryo, and from his description one would suppose the cells to be arranged in more definite tiers than they actually are. As to the rosette, my observations agree with STRASBURGER'S (18), that the cells composing it are enclosed

in walls and may divide after their formation. In one figure he shows each rosette cell divided into a group. According to him the embryo proper is formed from the group of cells lying between the tip cell (or cells) and the suspensors, and the tip cell is destroyed.

In comparing the proembryo of Cephalotaxus with that of its relatives, we find that it shows some resemblance to Taxus, but very little to Torreya. In Taxus, according to JAEGER (**8**), cell walls are not formed before the sixteen cell-stage at the earliest, but, as one would expect from the shape of the archegonium, there is no long and narrow cell at the apex of the embryo. In Torreya, according to Miss ROBERTSON (**17**) and COULTER and LAND (**6**), cell walls are formed in the four cell-stage. In *Torreya californica* the organized proembryo consists of an exposed rosette, a tier of four or six suspensors, and a "cluster" of tip cells (Miss ROBERTSON, **17**). In *T. taxifolia*, however, the proembryo entirely fills the archegonium with twelve or eighteen cells, all closed, and in this stage passes the winter (COULTER and LAND, **6**). Cephalotaxus, Taxus, and Podocarpus are the only conifers so far examined in which cell walls are not formed in the proembryo before the sixteen cell-stage.

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EXPLANATION OF PLATE I

FIGS. 1, 18–21 are in the text.

FIGS. 2–17 reduced one-third. Magnifications accordingly are now only two-thirds the number given.

FIG. 2. Body cell, stalk nucleus, and pollen tube nucleus in tip of a pollen tube that has reached the prothallium. April 21, 1903. ×670.

FIGS. 3–5. Sperm cells ready for fertilization. May 5 and 8, 1903. ×670.

FIG. 6. Neck cells seen from above. ×670.

FIG. 7. Neck cells pushed to one side. ×250.

FIG. 8. Ventral canal (*v*) and egg (*e*) nuclei in tip of archegonium. ×370.

FIG. 9. Archegonium showing ventral canal nucleus and two other nuclei below. ×150.

FIG. 10. Upper part of archegonium with egg (*e*) and ventral canal (*v*) nuclei. ×370.

FIG. 11. Upper part of archegonium with functional sperm cell approaching egg nucleus, and second sperm nucleus above. May 5, 1903. ×670.

FIG. 12. Archegonium and pollen tube just after fertilization; fusing nuclei in center and second sperm nucleus above. ×150.

FIG. 13. Fusing nuclei with sperm protoplasm on upper side. ×670.

FIG. 14. Spindle of first division of fusion nucleus; second sperm nucleus above. ×370.

FIG. 15. Two-celled proembryo with two nuclei above. May 8, 1903. ×250.

FIG. 16. Four-celled proembryo near base of archegonium. ×670.

FIG. 17. Eight-celled proembryo in base of archegonium. ×670.